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THERMOPHILIC BACTERIA.

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THE range of temperature at which it is possible for bacteria to grow is very great. Forster,¹ for example, proved that certain bacteria were capable of growth and multiplication at zero. These organisms were found by him in water, milk, soil, and street mud. When cultivated at this low temperature they still produced pigment, gas, light, etc. The apparently unfavourable external conditions did not overcome the vitality of the cells or interfere with the exercise of their specific properties.

For a large number of bacteria, 15°–20° C. is the optimum temperature, whilst for pathogenic bacteria the optimum temperature is blood heat. At temperatures above 40° C. relatively few micro-organisms grow, and at 50° C. most nonspore-bearing organisms quickly die. But there is undoubtedly a group of micro-organisms which can grow at temperatures above 50° C., and for which such high temperatures furnish the best conditions for their growth and development. Miquel's *Bacillus thermophilus*² is perhaps the most familiar example. It was found by him in air, rain water, river water, and in the alimentary canal of man and animals. It grows best at a temperature of from 65°–70° C.

Van Tieghem³ also describes a micrococcus which grew at 70° C., and Certes and Garrigon⁴ found, in the waters of Luchon, bacilli that could be cultivated at 64° C. The temperatures at which it is possible for bacteria to grow range therefore from zero to 70° C. Globig,⁵ on inoculating sterile milk and beef-broth with garden earth, obtained a growth of bacteria at 58° C. They were exclusively bacilli, and in many cases they produced spores after 24 hours' incubation at this temperature, potatoes being used as a culture soil. Globig obtained 30 bacteria, which grew at 58° C. At 68° C. only a few continued to grow. One bacillus grew at 68° C. and also at 15°–20° C. This was the greatest range of

¹ *Centralbl. f. Bakteriöl. u. Parasitenk.* bd. xii.

² *Ann. de Micrographie*, No. 1, 1888.

³ *Bull. Soc. Bot. de France*, tome xxviii.

⁴ *Compt. rend. Acad. sc.*, Paris, tome iii.

⁵ *Ztschr. f. Hyg.*, Leipzig, bd. iii.

temperature observed by Globig. As a rule, growth only began about 50° C. The bacilli were all isolated from garden soil. Experiments made with fæces and with water from various sources gave negative results. Globig, therefore, draws the conclusion that the thermophilic bacteria found by him in garden soil are not derived from the alimentary canals of animals or from water.

These are the main researches that we have been able to find on this subject, and they are more or less fragmentary. Beyond the fact of demonstrating their existence, comparatively little has been done in the study of this interesting group of organisms as a whole.

We undertook this investigation in order to determine whether the bacteria which grow at such high temperatures are widely distributed, and their number considerable; whether, in other words, they are to be looked upon as mere bacteriological curiosities, or as organisms which, from their number and distribution, probably fulfil some function in the economy of nature. We will first state what we have been able to determine with regard to their morphology, and finally touch upon their salient biological properties. To those organisms that grow best at very high temperatures we have applied the name of thermophilic bacteria, and this term will be employed in the course of this paper. The temperature we used in our experiments was attained in an incubator set at 60°–65° C., a temperature far removed from that at which the ordinary saprophytic bacteria will grow. By this means we avoided the introduction of any possible fallacy. If bacteria grew well at this high temperature, there would be no doubt about their being genuine thermophilic organisms.

The suggestion occurred that perhaps the spores of thermophilic organisms might be present in our culture soils, and that owing to their great resistance to heat they might survive the ordinary methods of sterilisation. We accordingly placed tubes containing sterilised gelatine, agar-agar, broth, potato and milk, in an incubator at 60°–65° C. Though the tubes were left for some days at this temperature, no growth appeared, nor did the milk curdle. We thus demonstrated, in the first instance, that our culture soils were free from thermophilic bacteria, and that they had been rendered absolutely sterile by the usual methods employed.

Control cultures were also made of well-known saprophytic bacteria, to be met with in various soils at ordinary temperatures. We selected *Proteus vulgaris*, *Proteus mirabilis*, *Proteus zenkeri*, *B. mesentericus fuscus*, *B. subtilis*, *B. mycoides*, and the *B. fluorescens liquefaciens*. These organisms showed no trace of growth, though left for 16 days in an incubator at 63°–65° C. We were thus able to settle two important points at the outset, viz. that our nutrient culture media were free from thermophilic bacteria or their spores, and that the thermophilic bacteria were organisms differing from those commonly met with in various soils at ordinary temperatures.

The first experiments were made with garden soil. We found that after inoculating nutrient agar-agar with garden soil, an abundant growth of bacteria was invariably obtained at 60°–65° C.

We endeavoured to determine, in the first place, whether these thermophilic bacteria are widely distributed in nature. The culture soils used for this purpose were gelatine, agar-agar, beef-broth, Koch's peptone water, and potatoes. We selected a variety of nutrient soils, in order to obtain as large a number of organisms as possible. Tubes containing these several culture media were inoculated directly with material from various sources. The tubes were then placed in an incubator at 60°–65° C. The results, concisely stated, were as follows:—

1. EXPERIMENTS WITH FÆCAL MATTER.

For the experiments, human fæces, mouse fæces, hen fæces, and horse dung were used. In all cases the results were positive. We obtained a good growth of bacteria at 60°–65° C. A sample of dysenteric fæces, which was sent to the laboratory, was also tested, and a growth of bacteria likewise obtained at the above temperature. It may be mentioned here that human saliva gave negative results.

2. EXPERIMENTS WITH SEWAGE MATTER.

We used for this purpose samples of Croydon sewage, before its passage to the sewage farm. Good growths of bacteria were obtained in the various culture media at 60°–65° C. The effluent, after leaving the sewage farm, was also found to contain thermophilic bacteria.

3. EXPERIMENTS WITH SAMPLES OF SOIL.

The surface soil taken from a London garden was found to contain numbers of thermophilic bacteria. Further, samples of soil taken at a depth of 2 in., 15 in., and 2 ft., gave in each case a growth of bacteria at 60°–65° C. We thus found those thermophilic organisms at a depth of 2 ft.

In a made London soil, owing to frequent disturbance, there must have been a considerable mixing of the deep and surface layers. We therefore made a series of experiments in a London suburb, in a garden where the soil had been undisturbed for years.

Samples of this undisturbed soil were taken from various depths by means of a Fränkel's earth borer. We examined the soil at a depth of 5 ft., 4 ft., 3 ft., 2 ft., and 1 ft., as well as the surface layers. All these samples gave a growth of bacteria at 60°–65° C.

The thermophilic bacteria are therefore not only widely distributed in the surface layers of the soil, but are to be found in the soil even at a depth of 5 ft.

4. EXPERIMENTS WITH SAMPLES OF WATER.

Thames water invariably gave a good growth of bacteria at 60°–65° C.

Thames mud taken from the bed of the river also gave a copious growth at the same temperature.

A sample of *sea water* taken at Broadstairs gave a positive result.

The results with London tap water were negative. The water from a thermal spring at Bath also gave a negative result. We were, however, only able to examine one sample of this water.

5. EXPERIMENTS WITH AIR DUST.

Rain which was collected during a shower gave no growth of bacteria at 60°–65° C.

The dust collected from the floor of the laboratory was found to contain thermophilic organisms, but the dust collected near the ceiling gave no growth.

The dust gathered from the London streets gave a good growth in every instance.

Finally, thermophilic organisms were also obtained from straw which had been placed in beef-broth, and incubated at 60°–65° C.

These thermophilic organisms are, therefore, most widely distributed—we found them in water, the soil, and air dust; in river mud, straw, and the dejecta of man and the lower animals.

Their distribution is wide, and they may almost be termed ubiquitous, as our experiments show.

It is interesting to note that these thermophilic organisms are not confined to the surface of the earth, as one would naturally expect, but that they extend to a considerable depth in the soil. We found them in the mud from the bed of the Thames, and in soil at a depth of 5 feet. Another point of interest is their very rapid growth at 60°–65° C. Agar tube cultures, placed in an incubator at 60°–65° C., gave a rapid and extensive growth in 15–17 hours, showing that this high temperature was one most favourable to the growth and development of these organisms.

The above experiments will be sufficient to show that these thermophilic bacteria are widely distributed, and that they are capable of existing under the most varied external conditions.

Our next endeavour was to isolate these organisms in pure cultures. The method we adopted was as follows. Nutrient agar-agar was liquefied, poured into Petri's culture dishes, and allowed to resolidify. An emulsion of the material to be investigated was made in distilled water. By means of a camel-hair brush, some of the emulsion was smeared over the surface of the agar-agar so as to form a very thin film. The culture dishes were then inverted, in order to prevent moisture running over the surface of the agar. The cultures so prepared were placed in a glass vessel, and covered with a bell jar. In the bottom of the glass receptacle some water was placed to prevent the agar becoming too dry. The vessel containing the plate cultures was placed in an incubator at 60°–65° C. In this way colonies of the thermophilic bacteria were obtained. From the colonies subcultures were made on various media, and their manner of growth was studied. The colonies developed on the plates very rapidly. After from 15–17 hours, good growths were generally obtained.

We may state here that all the thermophilic organisms examined by us were bacilli. We did not obtain any moulds, yeasts, cocci, spirilla,

or cladothrix forms. Further, all the organisms isolated by means of plate cultures, proved to be bacilli. We were also able to demonstrate that these bacilli do not all belong to one species, but that there are quite a variety of thermophilic bacilli. Representative forms, isolated from different sources were taken, and their morphology and growth on various culture media more closely studied.

In this way we were able to differentiate at least twenty different species. The number might easily have been extended, if we had carried our investigations further in this direction. The appended table will, however, suffice to give an idea of the variety of these forms that exist widely distributed in nature.

A bacillus, which was isolated from sea water, showed a very characteristic serpentine movement, which we only noticed in this form. A bacillus isolated from Thames mud produced a characteristic brick-red pigment on nutrient agar-agar.

As already stated, *all* the organisms isolated by us were bacilli.

The classical example of a thermophilic organism has hitherto been Miquel's *Bacillus thermophilus*. It might perhaps therefore be natural to assume that the number of bacilli capable of development at such high temperatures is very limited. Our experiments, however, speedily showed that there are quite a number of bacilli to which the term "thermophilus" may be employed. The twenty forms isolated by us did not exhaust the number. We must, therefore, regard the thermophilic bacteria as a large and important group of micro-organisms. Our observations quite confirmed those of Globig, as to the variety of species of the thermophilic bacilli. All the organisms that we carefully examined were spore-forming bacilli. We could distinguish four groups of bacilli, as regards spore formation:—

1. Those in which the spore is formed at or near the centre of the bacillus.

2. Those in which a round spore is formed at one end of the bacillus, giving the "drumstick" appearance.

3. Those with a large oval spore at one end of the bacillus.

4. Those with a small spore, not exceeding the diameter of the bacillus, at one end.

The spore formation took place at 60°–65° C., and the best media to study the production of spores were surface agar-agar and potato cultures.

A further point of interest was the motility of a number of the organisms. In hanging-drop specimens a number showed active movements, darting about with great rapidity. It was interesting also to note how long this motility lasted. A culture kept for three weeks at 60°–65° C., still contained actively motile bacilli—a further indication of the suitability of this temperature for the organisms. There was further a marked tendency to the formation of long chains of bacilli. These were often of great length, and stretched across the greater part of the field of the microscope. The chains were either straight, curved, or twisted

TABLE I.

Bacilli found in Thames Water.

No. of Bacillus.	Form and Arrangement.	Growth on Agar-Agar.	Growth on Sugar Agar.	Growth on Gelatine.	Growth in Broth.	Growth on Potato.	Growth in Milk.
1	Very long chains; ends round; end spore; motile.	Diffuse, greyish-white growth.	Slight surface growth.	Flocculent, greyish-white growth.	Cloudy, diffuse growth.	Cream-coloured growth.	No precipitation of casein.
2	Short chains; end round; end spore; motile.	Diffuse, dull white growth.	Very slight growth.	Ropy, viscous growth; liquefaction.	Very faint growth.	Little or no growth.	No precipitation of casein.
3	Curved chains; ends round; central spore; motile.	Cream-coloured, diffuse growth.	Do.	Diffuse growth.	General turbidity.	Yellowish-brown growth.	No precipitation of casein.

TABLE II.

Bacilli found in Surface Soil.

No. of Bacillus.	Form and Arrangement.	Growth on Agar-Agar.	Growth on Sugar Agar.	Growth on Gelatine.	Growth in Broth.	Growth on Potato.	Growth in Milk.
1	Long, delicate threads; central spore; motile.	No growth.	Slight surface growth.	No growth.	Surface pellicle.	Very slow growth; amber coloured.	No precipitation of the casein.
2	Short threads; central spore; motile.	Small dots; then diffuse.	Do.	Do.	Turbidity; no pel- licle.	Yellowish, glis- tening growth.	No precipitation of casein.
3	Long threads; end spore; non- motile.	White, diffuse growth.	Do.	Liquefaction.	Do.	Whitish film.	Curdles milk; preci- pitation of casein.
4	Long, slender threads; end spore; non-motile.	Whitish growth.	Do.	No liquefaction.	Do.	Yellowish-white.	Curdled.
5	Slender threads; central spore; non- motile.	White, granular growth.	Do.	Liquefaction.	Do.	Brownish-yellow.	Curdled, precipita- tion of casein; pink pigment.
6	Short, straight rods; rounded ends; end spore; motile.	Diffuse, dull white growth.	Very slight growth.	Pink, floeculent growth; lique- faction.	Diffuse cloudiness.	Brown, glisten- ing growth.	Curdled.
7	Plump bacillus; round ends; non- motile; central spore.	White, round patches.	Do.	Liquefaction.	Diffuse growth and pink pellicle.	Pale pink.	Do.
8	Short, straight rod; end spores; non- motile.	Slimy, diffuse growth	Do.	Floeculent growth; liquefaction.	Diffuse cloudiness.	Feeble growth; yellow pig- ment.	No action.

TABLE III.
Bacilli found in Street Dust.

No. of Bacillus.	Form and Arrangement.	Growth on Agar-Agar.	Growth on Sugar Agar.	Growth on Gelatine.	Growth in Broth.	Growth on Potato.	Growth in Milk.
1	Short rods; end spores; non- motile.	White film.	Not noted.	Slight, cloudy growth.	Diffuse cloudiness.	Honey-like growth.	Not noted.
2	Long, slender threads; end spores; motile.	Whitish film.	Do.	Turbidity; lique- faction.	Turbidity.	Transparent, whit- ish, shiny growth.	Curdled.
3	Short rods; end spores; motile.	No growth.	Do.	Turbidity; no lique- faction.	Diffuse cloudiness.	White crust.	Do.
4	Thin, slender rods; sometimes long threads; end spores; non-motile.	Arborescent growth.	Do.	Turbidity; lique- faction.	Do.	Slow, pink growth, with white edges.	Do.

The organisms were incubated at 60°-65° C. The gelatine was, of course, liquefied at these temperatures. Liquefaction of the gelatine was determined by removing the tubes to room temperature after a sufficient period of growth, and controlled by uninoculated gelatine, subjected for the same period to the same temperature.

in shape. There was no difficulty in staining the bacilli with the ordinary aniline dyes, nor in staining the spores by the usual methods. With regard to their growth on culture media, the ordinary soils gave good growths. Individual forms developed well on gelatine, agar, broth, potato, peptone water, and milk. The quickest growth was generally obtained by means of surface agar cultures. Differentiations in pigment production were most markedly developed in potato cultures—though the growth of the organisms was slower than on agar.

A certain number of the bacilli liquefied gelatine, some curdled milk, and others were specially characterised by the production of pigments. After having obtained pure cultures of the bacteria, it was of importance to prove their thermophilic properties. The term "thermophilic," strictly speaking, implies an organism that grows best at high temperatures; that can be cultivated from generation to generation at such high temperatures; and that does not grow, or at most feebly, at low temperatures. This test was applied to the bacilli we had isolated.

Subcultures were made, and placed first at 22° C., then at 37° C., and finally at 60°–65° C. No growth took place at 22° C., nor at 37° C., but on placing the tubes at 60°–65° C. a rapid growth once more took place. These experiments seemed clearly to prove that at or below blood heat the organisms did not develop, that temperatures above blood heat were necessary for their growth, and that 60°–65° C. was an optimum temperature. Also cultures that were carried on to the sixth generation continued to grow well at 65° C. The organisms could therefore with justice be called obligatory thermophilic bacteria.

A further series of experiments was made to determine the range of temperature at which it is possible for these bacteria to grow. For this purpose the temperature of an incubator was gradually raised from blood heat upwards. Fifteen organisms from various sources were tested. At blood heat no visible growth took place. One organism (a bacillus isolated from horse dung) began to grow at 40°–42° C. A slow growth began in 6 of the tubes at 50°–52° C. A slow growth started in 2 other tubes at 52°–55° C., and in two more instances commenced at 56° C. Four cultures remained undeveloped at 56° C., and their growth first began at 60° C. None of the organisms would grow at 75° C. Whilst we obtained a growth in some instances at 50°–52° C., the growth was slower, and not nearly so abundant as at 60°–65° C. At this latter temperature an almost instantaneous growth was obtained, and it evidently furnished the best conditions with regard to heat for the bacilli. The lower limit of growth for nearly all the bacilli appears to be 50° C., and the upper limit for the forms we isolated, 75° C. They, however, developed best between 55° and 65° C., giving a range of 10 degrees within which their growth was active. The organisms possess also a considerable resistance to the action of heat, as boiling for 10 minutes did not destroy them. The temperature at which it is possible for the thermo-

philic bacteria to grow lies so far above blood heat that we did not feel it necessary to test their pathogenic properties. The organisms isolated by Globig were found to be non-pathogenic. The thermophilic organisms are undoubtedly saprophytic organisms.

Micrococci were not detected, though Van Tieghem states that he found a coccus capable of development at 70° C. Miquel's *B. thermophilus* grew in broth at 60°–70° C., but did not grow in nutrient gelatine. The thermophilic bacteria isolated by Globig from garden soil began to grow, as a general rule, about 50° C. He found them in garden earth, but *not* in the dejecta of man, dogs, guinea-pigs, rabbits, pigeons, or mice. Horse dung, sewage water, Spree water, tap water, etc., gave negative results; and he concludes that the thermophilic bacteria of the soil are not derived from the alimentary tract of animals. Our experience was different. We found the organisms widely distributed in the soil; in the dejecta of man and the lower animals, and in water from various sources. On examining a virgin, sandy soil, Globig found that these bacteria disappeared at a depth of 8 inches. In our experiments we found them as deep as 5 feet in the soil. Lustig¹ describes a "red bacillus" which grew at room temperature, and also at 60° C., having therefore a remarkably wide range of growth. This bacillus produced no pigment when cultivated above blood heat. It is motile, and produces no endogenous spores. The bacillus reduces nitrates and develops nitrous acid. It, however, seems to be a rare form, as it was only isolated once from river water by Lustig.

There still remain some points of interest to be noted in connection with the general biology of the thermophilic bacteria we isolated. The inquiries made in this direction were by no means of an exhaustive nature; but sufficient indications were obtained that a more extended chemical investigation would lead to valuable and important results. The directions in which such investigations might be fruitful, will be sufficiently indicated by the following summary of our results.

A certain number of the bacilli liquefied gelatine, others did not. A certain number also produced a curdling of milk. A putrefactive decomposition took place in broth and agar cultures, characterised by a most repulsive smell, and the production of indol and sulphuretted hydrogen. The action of four organisms was tested on starch. Three of these produced no change in the starch. One bacillus (isolated from mouse faeces) converted the starch into sugar. The organisms did not produce any fermentation of sugar, nor did they thrive on soils containing sugar. Their growth on a sugar soil was very slow. It was evident that the presence of sugar did not favour, but was inimical to their growth. A good growth was obtained on urine in several instances. A bacillus isolated from horse dung produced a marked putrefactive decomposition of the urine. Cultures made in eggs gave, in one or two instances, H₂S gas.

¹ "Diagnostik d. Bakter. d. Wassers." 1893, p. 72.

Experiments were made with meat and with blood albumen. To one part of meat, four parts of water were added, and the whole sterilised in a flask. The blood albumen was prepared in the same way. The flasks were then inoculated with a small quantity of Thames mud, or of surface soil, and placed in the incubator at 60°–65° C. An active decomposition of the proteid matter took place, and a great development of H_2S , accompanied by an overpowering, nauseating odour. Indeed the smells developed on proteid soils by these thermophilic bacteria are things to be remembered.

A certain number produced pigments at these high temperatures, *e.g.* pink, yellow, or brown. A bacillus from Thames mud produced a brick-red pigment on agar-agar. The action of the organisms on cellulose was finally tested. A little meat broth or salt solution was added to straw or to cotton wool. The tubes were then inoculated with the micro-organisms. A bacillus from the soil, and one from Thames mud, grew well under these conditions, and gas was developed. Whilst not wishing to lay too great stress on these comparatively simple experiments, they sufficiently indicated that certain of the organisms, under favourable conditions, can probably induce a fermentative decomposition of cellulose. We may also add that straw suspended in broth gave a growth of bacteria at 60°–65° C.

Of four organisms tested, two grew anærobically, and one of these produced gas. The anærobic growth was, however, slow compared with the rapid, extensive surface growth of aërobic cultures of the same organism. Anærobic sugar cultures gave no fermentation of the sugar in the cases tested, and practically no growth of the bacteria. The sugar had an inimical action on their growth under both aërobic and anaërobic conditions.

These are the salient features of our investigation, succinctly stated.

We have then existing in nature a large number of bacteria, for which 60°–65° C. is an optimum temperature, and these organisms will not grow at or below blood heat. They grow best at 60°–65° C., and continue to grow at this temperature from generation to generation. At this high temperature they exercise perfectly all the functions common to saprophytic organisms. They liquefy gelatine, curdle milk, produce pigment, and convert starch into sugar. They produce an active decomposition of proteids, characterised by the development of H_2S , and a most repulsive smell; and they probably can produce a fermentation of cellulose. Their most marked property appears to be the decomposition of proteid bodies which they are able to effect.

It is indeed remarkable to find cell protoplasm existing and exercising all its functions at a temperature which is usually fatal to its life. One question naturally arises, and it is by no means an easy one to answer. How do these organisms exist under the conditions of temperature prevailing in this part of the globe? In the tropics the surface soil becomes greatly heated by the sun, and travellers tell us

that an egg can be cooked in the sand of the Sahara Desert. A badly conducting surface like sand becomes very hot when exposed to the sun's rays, and a dark surface soil absorbs a large quantity of heat. A superficial temperature of $68^{\circ}3$ C. has been observed in a sandy soil at the Cape of Good Hope. At Edinburgh, in a hole bored 2 feet into the solid rock, the minimum temperature was $4^{\circ}1$ C., and the maximum $11^{\circ}6$ C., giving a range of $7^{\circ}5$ C. for the year.

The conditions of climate existing in this country are not favourable to the growth of these thermophilic bacteria, and yet they are most widely distributed. It is apparent contradictions of this kind that stimulate investigation, and lead to valuable additions to our knowledge. Apart from climatic influences, it is most probable that these bacteria do find in nature conditions under which they are able to grow and develop. In this connection there is one point that seems to us well worthy of investigation, *i.e.*, the temperatures at which various fermentations take place, and the internal heat that is developed in the course of such fermentations. The maximum amount of nitrification of ammonia and its salts in the soil takes place at 37° C., and ceases at 55° C. We are familiar with the smoking of a manure heap. Manure, through the action of bacteria, undergoes a variety of fermentations, in the course of which considerable heat is developed. We have the most varied fermentations constantly going on in manure:—

1. The decomposition of fatty acids.
2. The decomposition of amido compounds, such as leucin and tyrosin.
3. The putrefaction of proteids.
4. The ammoniacal decomposition of urea.
5. The fermentations associated with the development of H_2S .
6. The cellulose fermentation. This fermentation occurs in every manure heap, and also in moist marshy soils. Tappeiner also found that bacteria contained in the intestines of cattle produced a cellulose fermentation, with a development of CO_2 and marsh gas. Very little is known about these bacteria. Schlosing found that he could start a very active fermentation of cellulose in manure by heating up to 42° and 52° C.¹ This cellulose fermentation is favoured by a high temperature, and is most active in summer.

7. The butyric acid fermentation, which takes place best at 40° C.

All these fermentations occur in manure, and the manure becomes sensibly hot. It may be that in the course of such fermentations, sufficient heat may be developed to start, at any rate, the growth of these bacteria.

When hay is packed moist, fermentation may occur, and so great a heat is developed within the stack that the hay may catch fire. The changes which go on in a moist haystack are also seen in the process of ensilage. If green fodder is stored in a silo, the mass becomes hot

¹ *Compt rend. Acad. d. sc.*, Paris, 1889.

from oxidation. Carbonic acid and other gases are developed. Mr. Warrington states that the temperature of the silo goes up to at least 140°–160° F. (60°–71° C.).

Ferdinand Cohn,¹ as the result of his investigations, comes to the conclusion that the "spontaneous combustion" of moist hay, etc., is due to a fermentation which is set up by micro-organisms. He found that on moistening cotton-wool waste the temperature rose, and reached in 24–30 hours a maximum of 67·2 C. The temperature then slowly fell within 6 days to the temperature of the air. A strong odour of trimethylamine was at the same time developed. Trimethylamine is developed frequently in the course of various bacterial fermentations. Cohn believes that the cause of this fermentation of the cotton-wool waste is a micrococcus. In any case the *sterilised* cotton-wool did not ferment, nor was there any rise of temperature. In the course of the fermentation oxygen is used up and much CO₂ gas is developed. If the supply of oxygen is cut off, no rise in temperature takes place. The process is, therefore, due to the action of aërobic bacteria, the germs of which are present in the cotton-wool, when imported from America. The bacteria, therefore, probably belong to the large and important group of soil organisms. To these organisms Cohn applies the name, "thermogenic bacteria."

In the Augsburg hothouses the flower-pots are heated by means of this cotton waste, which is packed round them. On moistening the cotton-wool a heat is developed sufficient for the growth of the plants.

The heat, therefore, developed in the course of various fermentations is great, going up to at least 160° F. (71° C.) and even higher, *i.e.* a temperature at which thermophilic bacteria would grow very well. It would be most valuable to have accurate data regarding the *internal* or localised heat developed in the course of various fermentations that are constantly taking place on a large scale in nature. As already pointed out, it is in many cases so considerable, that thermophilic bacteria may find, under such circumstances, conditions favouring not only their growth, but also the exercise of their special fermentative properties. It may be that they are also agents in the fermentation of cellulose, and the subject deserves further investigation, as well as the investigation of the amount of heat developed in this and other fermentations.

Whilst 55°–65° C. is the most favourable range of heat for these organisms, the possibility is not excluded that in some instances, at anyrate, their growth may be started at 40°–42° C.

It is difficult to believe that these thermophilic bacteria are simply "freaks," and that they only develop when a bacteriologist appears on the scene with an incubator. Their wide distribution, their good growth at these high temperatures, and their active fermentative properties, all point to their fulfilling some useful function in the economy of nature.

¹ *Berichte d. deutsch. botan. Ges.* 1893, bd. xi.



